US

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	10.07.2006

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
15658PCT00	

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK2005/000137	28.02.2005	01.03.2004

Applicant
MYCOMETER APS et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Wallentin, M Tel. +31 70 340-3991

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 15658PCT00	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/DK2005/000137	International filing date *(day/month/year)* 28.02.2005	Priority date *(day/month/year)* 01.03.2004
International Patent Classification (IPC) or national classification and IPC INV. C12Q1/24 C12Q1/04 C12Q1/34		
Applicant MYCOMETER APS et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 5 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27.12.2005	10.07.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Griffith, G Telephone No. +31 70 340-3191

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/DK2005/000137

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-21	as originally filed
Claims, Numbers	
1-47	received on 21.06.2006 with letter of 21.06.2006
Drawings, Sheets	
1/3-3/3	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-47
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-47
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-47
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:
D1 : WO 03/012397 A (MATSUSHITA SEIKO CO., LTD.) 13 February 2003 (2003-02-13)
D2 : US 5 811 251 A (A. HIROSE ET AL.) 22 September 1998 (1998-09-22)
D3 : EP 0 574 977 A (J. D. BERG) 22 December 1993 (1993-12-22)
D4 : US 4 871 662 A (E.ROSOV) 3 October 1989 (1989-10-03)

As D1 is written in Japanese the reasoning below and cited passages will be taken from the English language family member of D1, namely US 2004/219628, which is assumed to have the same content.

2 NOVELTY

2.1 Document D1 discloses (the references in parentheses applying to US2004/219628 as explained above) a collection unit ("a microorganism collecting chip") in which microorganisms present in a sample are trapped and subsequently detected by colour, fluorescence or luminescence, a microorganism collecting kit and a method of quantifying microorganisms using this microorganism collecting kit. (page 1, paragraph 1). The collection unit for the microorganisms includes a first filter for removal of contaminants with a pore size of 5-20 microns which allows the passage of microorganisms in the sample and a second filter with a pore size of 0.2-0.8 microns for trapping the microorganisms for detection (page 1, paragraph 5 and page 2, paragraph 31). (NB. The contaminants referred to in D1 are various types of debris which may interfere with the detection process (page 2, paragraph 30). The present application describes this type of debris as "larger particles" , whereas the contaminants are the microorganisms to be detected). The collection unit is provided with a suction filtration unit for applying negative pressure to the collection unit thereby facilitating flow of the sample through the filters (page 1, paragraphs 13-14 and page 3, paragraph 40). Quantification of microorganisms is provided by trapping said microorganisms on a filter followed by staining (page 2, paragraph 19).

Alternatively quantification of the microorganisms trapped on the collection filter is achieved by differential colouration of the various classes of microorganisms, both living and dead, by application of different colorant compounds (page 3, paragraph 45 and page 6, paragraph 79). Furthermore viable cells are detected by colouring using compounds which react with enzymes present in the microbial cells to form coloured or fluorescent products. Various examples include 4- methylumbelliferone derivatives (page 6, paragraph 81).
When a test sample is a solid sample such as foodstuffs including meat and vegetables, it is homogenised to prepare a liquid specimen (page 5, paragraph 64).

Various additives can be added to the sample liquid. Surfactants for releasing microorganisms which may be adhered to debris in the sample, polypeptone for maintaining the activity of the microorganisms or a polyhydric alcohol for preventing deactivation of the microorganisms or decay of luminescence caused by drying of the filter surface (page 7, paragraph 83).
The difference between the present application (PA) and D1 is that instead of measuring the microorganisms trapped on the filter surface by staining, the liquid vehicle surrounding the microorganisms can be used as the object for the measurement and thus a relatively simple measurement apparatus can be used which does not necessitate means for optical measurement which focus on the filter surface. As this feature is not disclosed in D1, the PA may be considered to be novel over D1.

D3 discloses (the references in parentheses applying to this document) "a direct method for detecting very low levels of coliform contamination in products for human consumption comprising contacting the microorganisms with a methylumbelliferone substrate. The substrate is hydrolysed into methylumbelliferone by an enzyme given off by the microorganisms. The methylumbelliferone is detected by its fluorescence, either in solution or ...." (abstract). Furthermore "The general procedure for the detection of TC (Total coliform) or FC (Fecal coliform) activity ..... is as follows: (a) the sample is concentrated by passing it through a membrane filter (0.2 micrometers to 0.80 micrometers pore size);
(b) the microorganisms which are retained with the filter are aseptically placed in contact with a sterile medium containing the appropriate 4-MU-substrate; and the

resulting fluorescence is measured and utilized as the rate of production of fluorescent product in the liquid medium associated with the sample determined at regular intervals over about fifteen minutes using a fluorescence detecting meter (column 6, line 52-column 7, line 8).
In contrast to D3, the present application does not require the filter to be removed from the filter device in order to detect the microorganisms trapped thereon.
Consequently the PA is considered to meet the criteria of Article 33(1) PCT, because the subject-matter of independent claims 1, 46 and 47 are novel in the sense of Article 33(2) PCT.

3. INVENTIVE STEP

D1 is considered to be the closest prior art (CPA) (see 2.1 above). The difference between the present application (PA) and D1 is that instead of measuring the microorganisms trapped on the filter surface by staining, the liquid vehicle surrounding the microorganisms can be used as the object for the measurement. The problem to be solved may be considered to be how to avoid the use of means for optical measurement which focus on the filter surface. The solution to this problem would be to use a system which allows for relatively simple measurement apparatus for detection of a colour change in the medium surrounding the microorganisms. As none of the cited prior art documents suggest this solution could be provided without first removal of the filter with trapped microorganisms from the filter device, it would not be obvious for the man skilled in the art to arrive at this solution. Consequently independent claims 1, 46 and 47 are considered as involving an inventive step (Article 33(3) PCT).

4. Claims 2-45 are dependent on claim 1 and as such also meets the requirements of the PCT with respect to novelty and inventive step.

5. The subject matter of claims 1-47 meets the requirements of Art. 33(4) PCT, having regard to industrial application.

CLAIMS

1. A sample preparation method for a medium suspected of containing contaminants, the method comprising a) passing a known volume of said medium through a filter from an influent side to an effluent side in a filter device thereby concentrating the contaminants on the influent side of the filter in the filter device, b) contacting the influent side of the filter in the filter device with a liquid vehicle containing at least one substrate that through interaction with the contaminants each produces a detectable moiety, c) and allowing the substrate to interact with the contaminants on the influent side of the filter in the filter device for a period of time, which is sufficient to allow the detectable moiety to be detected in the liquid vehicle.

2. The method according to claim 1, wherein, prior to step a, the medium is passed through a prefilter that does not retain the contaminants, but retains larger particles.

3. The method according to claim 1 or 2, the contaminants are selected from the group consisting of bacteria; fungi, such as filamentous fungi and yeast; algeae; protozoans; spores from bacteria; fungal spores; and pollen, and fragments thereof.

4. The method according to any one of the preceding claims, wherein the medium is a liquid medium.

5. The method according to claim 4, wherein the liquid medium is selected from the group consisting of environmental water, drinking water, hot water, industrial water, process water, cleaning in place water, a liquid extract of a solid material, a suspended or solubilised surface sample, and liquid industrial products such as cosmetics, pharmaceuticals, and foodstuffs.

6. The method according to claim 4-5, wherein the viscosity of the liquid medium is reduced prior to step a.

7. The method according to claim 6, wherein viscosity is reduced by means of dilution or by means of treatment with a chemical agent such as a solubility enhancing agent or a detergent.

8. The method according to any one of claims 1-3, wherein the medium is a gaseous medium.

9. The method according to claim 8, wherein the gaseous medium is air, such as air from a sterile facility, a laminar air-flow device or environmental air.

 AMENDED SHEET

10. The method according to any one of the preceding claims, wherein the filter has a pore size small enough so as to retain substantially all contaminants in the medium.

11. The method according to claim 10, wherein the filter has a pore size large enough to let the detectable moiety pass through the filter.

12. The method according to claim 11, wherein the pore size is at most 20 μm.

13. The method according to claim 11 or 12, wherein the pore size is at least 0.1 μm.

14. The method according to any one of the preceding claims, wherein the at least one substrate produces the detectable moiety by being cleaved by an enzyme that is characteristic for the contaminants.

15. The method according to claim 14, wherein the enzyme is selected from the group consisting of carbohydrases, proteases, lipases, esterases, amidases, sulfatases, nucleases and phosphatases such as alkaline phosphatase.

16. The method according to claim 14 or 15, wherein the enzyme is expressed constitutively by microorganisms.

17. The method according to any one of claims 14-16, wherein the at least one substrate is a fluorogenic or chromogenic substrate producing blue, green and red fluorescent products as the detectable moiety.

18. The method according to any one of claims 14-17, wherein the at least one substrate is selected from the group consisting of 5-bromo-4-chloro-3-indolyl phosphate disodium salt; 9h-(1,3-dichloro-9,9-dimethylacridine-2-one-7-yl) phosphate ammonium salt; fluorescein diphosphate tetraamonium salt; a methylumbelliferyl derivative such as 6,8-difluoro-4-methylumbelliferyl phosphate, 4-methylumbelliferyl phosphate dicyclohexylammonium salt trihydrate, 4-methylumbelliferyl phosphate free acid; 4-methylumbelliferyl phosphate dilithium salt, 4-methylumbelliferyl-β-N-acetylglucosaminide, and trifluoromethylumbelliferyl phosphate; salts of 4-nitrophenyl phosphate; and resorufin phosphate.

19. The method according to any one of claims 14-18, wherein the detectable moiety is detectable in an amount of at the most 100 picomoles, preferably at the most 50 picomoles, more preferably at the most 20 picomoles and even more preferably at the most 10 picomoles and most preferably at the most 1 picomoles.

20. The method according to any one of the preceding claims, wherein at least two substrates are used that produce detectable moieties providing signals that can be combined into one single measured signal value.

21. The method according to any one of claims 1-20, wherein at least two substrates are used that produce detectable moieties providing distinguishable signals.

22. The method according to any one of the preceding claims, wherein the contaminants are viable microorganisms.

23. The method according to any one of the preceding claims, wherein the amount of substrate in the liquid vehicle does not limit the rate of production of the detectable moiety.

24. The method according to claim 23, wherein the rate of production of the detectable moiety is a function of the quantity of contaminants in the known volume of the medium.

25. The method according to claim 24, wherein the function is linear.

26. The method according to any one of the preceding claims, wherein several different known volumes of the medium are each passed through a filter in step a, so as to ensure that at least one of the volumes contains a suitable number of contaminants.

27. The method according to any one of the preceding claims, wherein the filter is part of a closed, sterile filter device.

28. The method according to claim 27, wherein the closed, sterile filter device is disposable.

29. The method according to claim 27 or 28, wherein the closed, sterile filter device integrates the filter and a filter housing into one irreversibly closed structural unit.

30. The method according to any one of claims 27-29, wherein the longest cross-sectional axis of the closed, sterile filter device does not exceed a length of 10 cm.

31. The method according to any one of the preceding claims, wherein the interaction in step c is terminated by interrupting the contact between the substrate and the contaminants.

32. The method according to claim 31, wherein interruption is obtained by evacuating the liquid vehicle from the filter device while retaining the contaminants in the filter device.

33. The method according to claim 32, wherein the liquid vehicle is evacuated from the filter device in the direction from the influent to the effluent side of the filter.

34. The method according to claim 33, wherein evacuation is obtained by applying an elevated pressure on the influent side of the filter or by applying a lowered pressure on the effluent side of the filter.

35. The method according to any one of claims 1-30, wherein the interaction in step c is terminated on the filter or wherein the interaction is not terminated.

36. The method according any one of the preceding claims, comprising, after step c, a further step d) that entails detecting, quantitatively or qualitatively, the detectable moiety in the liquid vehicle and correlating the detection of the moiety to the amount or presence of contaminants in the sample.

37. The method according to claim 36, wherein detection in step d is performed by measuring fluorescence characteristic of the detectable moiety.

38. The method according to claim 37, wherein the fluorescence in step d is measured directly on the liquid vehicle without an interruption of the contact between the liquid vehicle and the contaminants.

39. The method according to any one of claims 36-38, wherein the correlation in step d comprises the use of a pre-determined standard curve that expresses the relationship between the amount of contaminants and the amount of the detectable moiety under standard conditions.

40. The method according to any one of claims 36-39, wherein detection is performed in a microtiter system.

41. The method according to any one of the preceding claims, wherein the contaminants are subjected to a signal-enhancing influence, either prior to step a or in step b.

42. The method according to claim 41, where the signal-enhancing influence increases the overall sensitivity in a subsequent detection or favours subsequent detection of specific types of contaminants, or reduces detection of specific types of contaminants.

43. The method according to claim 41, wherein the signal-enhancing influence is selected from an enzyme-enhancing substance, a selective temperature or temperature range, a selective pH, a selective salt concentration, a non-selective growth-enhancer, and a selective growth-enhancing substance.

44. The method according to any one of the preceding claims, wherein step a is preceded by an incubation of the medium.

45. The method according to claim 44, wherein the incubation entails

- treatment with an enzyme inducing substance thereby enhancing the detection of the detectable moiety, and/or
- subjecting the medium to a selective substance for yeast, fungi or bacteria, and/or
- subjecting the medium to a non-selective growth-enhancer for microorganisms, and/or
- subjecting the medium to a substance capable of extracting cellular enzymes.

46. A kit for determination of contaminants in a medium, the kit comprising

- at least one sterile filter device comprising a filter with a pore size sufficiently small to retain the contaminants on the filter's influent side,
- means for passing a known volume of medium through the filter,
- at least one agent that upon interaction with the contaminants will release a detectable moiety, the amount of which can be correlated with the amount of contaminants that have interacted with the agent, and
- instructions that sets forth steps for a) obtaining a known volume of medium and passing it through the sterile filter device, b) contacting the influent side of the filter in the filter device with the agent, c) allowing the agent to interact with contaminants that might be on the influent side of the filter in the filter device, and d) quantitatively detecting the detectable moiety.

47. Use of a closed, sterile filter device as a reaction vessel for a reaction between contaminants retained in the device and a substrate that releases a detectable moiety when contacted with the contaminants.